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Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study

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22 0. Abstract

23 The effect of hydraulic retention time (HRT) and seasonality on the removal efficiency of 26
24 organic microcontaminants from urban wastewater was studied in two pilot high-rate algal ponds
25 (HRAPs). The targeted compounds included pharmaceuticals and personal care products, fire
26 retardants, surfactants, anticorrosive agents, pesticides and plasticizers, among others. The pilot
27 plant, which was fed at a surface loading rate of 7-29 g of COD m⁻² d⁻¹, consisted of a
28 homogenisation tank and two parallel lines, each one with a primary settler and an HRAP with a
29 surface area of 1.5 m² and a volume of 0.5 m³. The two HRAPs were operated with different HRTs
30 (4 and 8 d). The removal efficiency ranged from negligible removal to more than 90% depending on
31 the compound. Microcontaminant removal efficiencies were enhanced during the warm season,
32 while the HRT effect on microcontaminant removal was only noticeable in the cold season. Our
33 results suggest that biodegradation and photodegradation are the most important removal pathways,
34 whereas volatilization and sorption were solely achieved for hydrophobic compounds (log K_{ow}>4)
35 with a moderately high Henry's law constant values (11-12 Pa m⁻³ mol⁻¹) such as musk fragrances.
36 Whereas acetaminophen, ibuprofen and oxybenzone presented ecotoxicological hazard quotients
37 (HQs) higher than 1 in the influent wastewater samples, the HQs for the effluent water samples
38 were always below 1.

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44 **Keywords:** *emerging organic contaminants; microalgae; high-rate algal pond; photodegradation;*
45 *biodegradation; volatilization.*

46 **1. Introduction**

47 Emerging organic contaminants (EOCs) include a wide range of compounds belonging to different
48 chemical classes, such as pharmaceuticals, personal care products, plasticizers, flame retardants,
49 surfactants, and certain pesticides, among others, the ecotoxicological effects of which are relatively
50 unknown [1]. Since conventional wastewater treatment plants (WWTPs) are not designed to remove
51 emerging and related contaminants, many of these compounds occur at different concentrations in
52 natural water bodies [2], where they may exert ecotoxicological effects at relatively low
53 concentrations [3, 4]. Although some of the compounds have been proposed for inclusion on
54 regulatory lists of contaminants (European Commission, 2006), there is relatively little information
55 on the ecotoxicological effects of complex mixtures at environmental levels, and, to date, they have
56 not been regulated [1]. Known environmental effects of some EOCs include the reduction of
57 macroinvertebrate diversity in rivers [3], behavioural changes in mosquito fish [4] and reproductive
58 disruption in fish [5], among others. Due to the difficulty of assessing the effects of EOCs on
59 ecosystems, the use of hazard quotients (HQs) based on the chemical composition of water samples
60 and tabulated predicted non-effect concentrations (PNECs) for different aquatic organisms has been
61 postulated as a good screening strategy [6].

62 Microalgae-based wastewater treatment technologies such as high-rate algal ponds (HRAPs) have
63 received considerable attention in recent years due to the resource recovery of algal biomass, for use
64 as fertilizer, protein-rich feed or biofuel, and a high-quality effluent (treated wastewater)[7]. HRAPs
65 are shallow raceway reactors in which microalgae and bacteria grow in symbiosis. In such systems,
66 organic matter is degraded by heterotrophic bacteria, which consume oxygen provided by
67 microalgal photosynthesis; therefore, no aeration is needed [8]. Although the capability of
68 microalgae wastewater treatment systems to remove nutrients, heavy metals, bacteria, and
69 helminthic eggs has been studied since the 1950s, few studies have focused on the removal of
70 organic contaminants, namely, phenolic compounds, surfactants, biocides and polycyclic aromatic

71 hydrocarbons [9-12]. Indeed, no attention has been paid to the effectiveness of HRAPs for
72 removing EOCs of environmental concern.

73 The removal of EOCs by conventional activated sludge WWTPs has been widely studied, but the
74 effectiveness of HRAPs for removing EOCs from wastewater has not yet been addressed. There is
75 only one study dealing with HRAPs' capacity to remove tetracyclines, and it was performed at
76 laboratory-scale with synthetic wastewater [13]. Other studies dealing with microalgae's capacity to
77 remove organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), biocides (e.g.
78 organotin compounds), surfactants and phenolic compounds, suggest that microalgae-based
79 wastewater technologies may remove microcontaminants by both abiotic (sorption, volatilization or
80 photodegradation) and biotic (biodegradation, microalgae uptake or metabolization) processes [14-
81 16].

82 The aim of this study was to evaluate for the first time, the effect of hydraulic retention time (HRT)
83 and ambient temperature / sunlight irradiation (seasonality) on the removal efficiency of 26 EOCs
84 in two HRAP pilot plants fed with real urban wastewater. The selected compounds were high
85 production volume chemicals (e.g. fire retardants, surfactants, anticorrosive agents, pesticides,
86 plasticizers, pharmaceuticals and personal care products, among others). Finally, aquatic risk
87 assessment was performed based on the concentrations of the detected EOCs in the influent and
88 effluent water samples, and the listed EC50 values for *Daphnia magna*.

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90 **2. Material and Methods**

91 *2.1. Chemicals and reagents*

92 Gas chromatography (GC) grade (Suprasolv) hexane, methanol, and ethyl acetate were obtained
93 from Merck (Darmstadt, Germany). Analytical-grade hydrogen chloride was obtained from Panreac
94 (Barcelona, Spain). Caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate, oxybenzone,

ketoprofen, hydrocinnamic acid, 5-methylbenzotriazole, naproxen, carbamazepine, galaxolide, benzothiazole, diclofenac, methylparaben, benzotriazole, tonalide, OH-benzothiazole, tributyl phosphate, tris(2-chloroethyl) phosphate, triphenyl phosphate, triclosan, cashmeran, octylphenol, diazinon, celestolide, atrazine, bisphenol A, 2,4-D, atrazine D5, mecoprop D3, tonalide D3 and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). Trimethylsulfonium hydroxide (TMSH) was obtained from Fluka (Buchs, Switzerland). Strata-X polymeric SPE cartridges (200 mg) were purchased from Phenomenex (Torrance, CA, USA) and the 0.7 μm glass fibre filters (\varnothing 47 mm) were obtained from Whatman (Maidstone, UK).

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104 2.2. Description of the HRAP pilot plant

The experimental set-up was located outdoors at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya-BarcelonaTech, Spain). The system has been operated since March 2010. The microalgae production system was composed of a screening pre-treatment and two identical parallel lines, each one equipped with a primary settler, a pilot high-rate algal pond and a final settler for biomass separation (Fig. 1). Paddle wheel was set at 5 rpm giving mixed liquor with a linear velocity of recirculation of 11 cm s^{-1} , enough to ensure complete mixing. Urban wastewater was pumped from a municipal sewer to a homogenisation tank (1.2 m^3), which was continuously stirred to avoid solids sedimentation. From there, the wastewater was pre-treated and conveyed to each line. The primary treatment included a settler with an internal diameter of 0.3 m, a total height of 0.4 m and an effective volume of 7 L that was operated at an HRT of 0.9 h. Primary effluent from the settlers was pumped to the HRAPs by means of peristaltic pumps. The experimental HRAPs were PVC raceway ponds equipped with a paddle wheel for stirring the mixed liquor (Fig.1). The two HRAPs had a nominal volume of 0.47 m^3 , a surface area of 1.54 m^2 and a water depth of 0.3 m, and they were operated simultaneously with different HRTs (4 and 8 days corresponding to 117.5 and 58.8 L d^{-1} respectively). The final settlers for biomass separation had an

120 internal diameter of 0.15 m, a total height of 0.3 m and an effective volume of 3.5 L that were
121 operated at an HRT of 0.7 and 1.4 h for the HRAP set at 4 days HRT and 8 days, respectively. Note
122 that these settlers were only used for biomass separation, which was not recycled back to the
123 HRAPs.

124

125 *2.3. Sampling strategy*

126 Two sampling campaigns were carried out, one in July 2013 (warm season) and the other in
127 December 2013 (cold season). In each campaign, influent and effluent grab samples were collected
128 from both HRAPs at the same time each day (9:00 am) for a period of 10 days (n=8), from Monday
129 to the Wednesday of the next week, Saturday and Sunday were not sampled. The samples were
130 collected in the primary effluent from the settler and at the effluent from both HRAPs (Fig. 1). No
131 rainfall events were recorded at any time during the sampling period. All water samples were
132 collected in 1000 mL amber glass bottles, which were transported under refrigeration to the
133 laboratory, where they were stored at 4 °C until analysis. The sample holding time was less than 12
134 hours.

135

136 *2.4. Analytical procedures*

137 Conventional wastewater quality parameters, including ammonium nitrogen ($\text{NH}_4\text{-N}$), total
138 suspended solids (TSS) and chemical oxygen demand (COD), were determined using the Standard
139 Methods (APHA, 2001). Onsite measurements of water temperature, dissolved oxygen (DO) and
140 pH were taken using a Checktemp-1 Hanna thermometer, an Eutech Ecoscan DO6 oxygen meter
141 and a Crison pH-meter, respectively.

142 For each campaign, 2 well-mixed 25 mL samples from each HRAP were examined by light
143 microscopy and the predominant microalgae were identified and quantified. Microalgae genus were
144 identified from classical specific literature [17, 18].

145 All water samples were filtered and processed as previously reported (Matamoros and Bayona,
146 2006). A 100 mL sample was spiked with 50 ng of a surrogate standard (atrazine D5, mecoprop D3,
147 tonalide D3, and dihydrocarbamazepine). The spiked sample was percolated through a previously
148 activated polymeric solid-phase extraction cartridge (200 mg Strata X). Elution was performed with
149 10 mL of hexane/ethyl acetate (1:1). The eluted extract was evaporated under a gentle nitrogen
150 stream until ca. 100 μ L remained, at which point 20 ng of triphenylamine was added as an internal
151 standard. Finally, the vial was reconstituted to 300 μ L with ethyl acetate.

152 The TSS collected in the glass fibre filters (0.7 μ m) were processed according to a previously
153 reported analytical method [19]. Briefly, the filters were freeze-dried and extracted in an ultrasonic
154 bath with hexane/acetone (3:1) for 15 minutes. The extracts were then further processed as water
155 samples.

156 Methylation of the acidic carboxyl group was performed in a hot GC PTV injector (270 $^{\circ}$ C) by
157 adding 10 μ L of TMSH solution (0.25 mol L⁻¹ in methanol) to a 50 μ L sample before injection.
158 Derivatized samples were analysed into a Bruker 450-GC gas chromatograph coupled to a Bruker
159 320-MS triple quadrupole mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA) fitted
160 with a 20 m \times 0.18 mm, 0.18 μ m film thickness Sapiens X5-MS capillary column coated with 5%
161 diphenyl 95% dimethyl polysiloxane from Teknokroma (Sant Cugat del Vallès, Spain) operated in
162 the multiple reaction mode (MRM). Validation of the analytical methodology has been described
163 elsewhere [20]. The limit of detection (LOD) and limit of quantification (LOQ) of the analytical
164 methodology were determined (using ultra-pure water) based on the mean background noise plus 3
165 or 10 times the standard deviation of the background noise, respectively. The LOD and LOQ ranged
166 from 1 to 40 ng L⁻¹ and from 3 to 80 ng L⁻¹, respectively. Recoveries and repeatability were always

167 higher than 80% and lower than 20%, respectively.

168

169 2.5. Data analysis

170 The removal efficiencies of conventional water quality parameters and EOCs were calculated as
171 follows (equation 1):

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$$174 \text{ Removal} = \frac{1}{n} \sum_{i=1}^n \frac{C'a - (Ci - Ci \times \frac{EVR}{HLR})}{C'a} \times 100 \quad (\text{Equation 1})$$

175

176 where $C'a$ is the average concentration of a selected compound in the HRAP influents in each
177 sampling campaign, Ci is the concentration in the HRAP effluents on each sampling day, and n is
178 the number of samples collected per sampling campaign ($n=8$). HLR are 83 or 43 $\text{L m}^{-2} \text{d}^{-1}$ at a HRT
179 of 8 and 4 days, respectively. Evaporation rates (EVRs) are 21 and 9 $\text{L m}^{-2} \text{d}^{-1}$ in warm and cold
180 season, respectively calculated from Turc's equation.

181

182 The experimental results were statistically evaluated using the SPSS v.13 package (Chicago, IL,
183 USA). According with the data set size, non-parametric statistics were applied. The comparison of
184 means was conducted by means of the Kruskal–Wallis test. Spearman's coefficients were used for
185 correlations between variables (physicochemical parameters, removal efficiencies and influent
186 concentration). Significance was defined as $p < 0.05$.

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191 3. Results and Discussion

192 3.1 Assessment of conventional water quality parameters

193 The HRAPs' performance was monitored in warm and cold seasons (Table 1). COD, TSS and NH₄-
194 N concentrations in the primary settlers' effluents were within the typical range of a conventional
195 primary effluent [21]. Note that DO concentration was quite high for a primary effluent due to
196 wastewater stirring in the homogenisation tank. The actual surface organic loading rates (OLRs)
197 applied to the HRAPs were, on average, 13 and 26 g total COD m⁻² d⁻¹ (HRT=8 and 4 d
198 respectively) in the warm season and 29 and 58 g total COD m⁻² d⁻¹ (HRT=8 and 4 d respectively)
199 in the cold season.

200 The microalgae present in the HRAPs were mostly species of the Phylum *Chlorophyta*, and varied
201 depending on seasonal conditions. In the warm season, predominant algae species were
202 *Stigeoclonium* sp. (filamentous algae); diatoms, *Chlorella* sp. and *Monoraphidium* sp. In the cold
203 season, predominant algae species were *Chlorella* sp.; diatoms, *Stigeoclonium* sp. In both
204 campaigns, the number of species was higher in the HRAP set at HRT of 8 days.

205 In the present study, mixed liquor TSS was analyzed as an indicator of biomass concentration in the
206 HRAP. Note that biomass in such type of systems corresponds to microalgae as well as bacteria, and
207 the proportion of each cannot be easily estimated with conventional well-established kinetics and
208 thermodynamic data for heterotrophic cultures. However, previous studies based on microscope
209 observations and the linear correlation between chlorophyll *a* and TSS suggest that the percentage
210 of microalgal biomass in HRAPs is higher than 80-90% [22-24]. Average microalgal biomass
211 concentration and production were clearly higher in the warm season than in the cold season in
212 relation with the higher solar radiation (Table 1). Biomass concentration was slightly higher in the
213 HRAP with a HRT 8 days where lower flow-rate gave place to less biomass wash-up, as already
214 observed in other studies [22, 25, 26]. Despite this, biomass production was higher in the HRAP
215 operated at a HRT of 4 days, in these systems biomass production usually increases inversely with

216 the HRT [27].

217 Biomass production values are in accordance with those previously reported [28]. De Godos et al.
218 [29] observed a biomass production of 21.3-27.7 g TSS m⁻² d⁻¹ in summer (average solar radiation
219 of 6774 W d⁻¹ m⁻²) for HRAPs operating at 10 HRT fed with diluted swine manure. In winter period
220 (solar radiation of 1785 W d⁻¹ m⁻²) biomass production decreased to 5.7-6.1 g TSS m⁻² d⁻¹. García et
221 al. [23] using the same HRAPs as in the present study reported a production between 12.7 and 14.8
222 m⁻² d⁻¹. The HRAPs' performance (Table 1) was consistent throughout the experimental period, with
223 removal efficiencies similar to those reported in previous studies for this pilot plant [8] and others
224 previously reported [28]. COD removal was moderate (66-85%) and its removal in these systems
225 depends on influent concentration because background concentration remains around 50-70 mg L⁻¹
226 [23]. Up to 99% of NH₄-N was removed in the HRAPs in the warm season at both HRTs, whereas
227 the removal rate was lower and different at both HRTs (90 vs 98%) in the cold season. Hence,
228 environmental conditions (i.e. temperature and solar radiation) played an important role in NH₄-N
229 removal, whereas HRT was only relevant in the cold season. Mechanisms for nitrogen removal have
230 been studied in detail in the past and the most predominant include volatilisation, biological uptake
231 and nitrification [24, 28]. NH₄-N removal values were similar to those found in the literature. De
232 Godos et al. [29] found in summer a COD and NH₄-N removal of 76 and 96% respectively, whereas
233 in winter those removal decreased to 57 and 92% for HRAPs operating at a HRT of 10 days.
234 Sutherland et al. [30] found a decrease on NH₄-N removal between summer (77%) and winter
235 (53%) in a HRAP operating at a HRT of 4 and 9 days respectively.

236

237 3.2. Occurrence and removal efficiency of EOCs

238 For this study, we selected the emerging contaminants with the highest concentrations that are most
239 often detected in raw wastewaters [31]. The concentration of EOCs in HRAP influent (primary
240 effluent) ranged from undetected to 24 µg L⁻¹ (Fig. 2). Caffeine, acetaminophen and ibuprofen were

usually detected at concentrations higher than $9 \mu\text{g L}^{-1}$, in keeping with the reported literature [32].

Although the influent COD showed a high seasonal variability (Table 1), the concentration of the studied EOCs showed no statistically significant seasonal difference ($p=0.84$). This may be explained by the presence of a primary treatment that favours a more constant influent wastewater in terms of quality, as can be seen in the low variability of the EOC concentrations (Fig. 2).

Table 2 shows the removal efficiencies of the target EOCs. They can be classified into four groups in accordance with the corresponding overall average removal efficiency in the HRAPs: high removal ($>90\%$: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate and hydrocinnamic acid), moderate-to-high removal (from 60% to 90%: oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate, triclosan, bisphenol A and octylphenol), moderate-to-low removal (from 40 to 60%: diclofenac, benzotriazole, OH-benzothiazole, triphenyl phosphate, cashmeran, diazinon, benzothiazole, celestolide, 2,4-D and atrazine) and poor or no removal ($<40\%$, carbamazepine, methyl paraben, tris(2-chloroethyl) phosphate).

Taking into account the configuration of the HRAPs, the most relevant removal processes that may occur in these systems can be biodegradation, photodegradation, volatilization and sorption to microalgae biomass. Uptake by microalgae is an important removal process, and it was assessed by analysing the occurrence of EOCs in the TSS (solids retained in the filters). Table 1 in the Supplementary Material (SM) shows that the most abundant compounds in the biomass (mostly microalgae) were the most hydrophobic ones, such as galaxolide and tonalide ($\log K_{ow}>5$). Hence, HRAPs may remove hydrophobic compounds by sorption, similarly to other wastewater treatment technologies such as constructed wetlands (CWs) and activated sludge systems [33, 34]. Despite the higher concentration of TSS in the HRAPs during the warm season, due to the greater biomass production and evaporation losses (Table 1), the concentration of musk fragrances in the TSS was higher in winter. Therefore, it may be postulated that the increase in biomass (microalgae,

heterotrophs and non-photosynthetic autotrophs organisms) improved the biodegradability of these compounds or that the higher sunlight irradiation and temperatures in warm season improved the volatilization rates. This is in keeping with the moderate biodegradability found for musk fragrances (>75%) in lab-scale activated sludge reactors [35] and the tabulated high Henry's law constants (a measure of air-water partitioning) for musk fragrances (11-12 Pa m⁻³ mol⁻¹). The occurrence of most of the studied EOCs in the filters was below their LOD. This may be due to the fact that these EOCs were not uptaken by microalgae or because they were removed by microalgal metabolism. A microalgae removal effect due to the release of exudates likewise cannot be disregarded [36]. In fact, it has been proved that the consortia of cyanobacteria/microalgae and bacteria can be efficient in detoxification of organic and inorganic pollutants, and removal of nutrients from wastewaters, compared to the individual microorganisms. Cyanobacterial/algal photosynthesis provides oxygen and organic exudates that serves to the pollutant-degrading heterotrophic bacteria [37].

The overall average removal efficiencies of the studied EOCs were plotted against their physicochemical properties (log K_{ow}, molecular weight (MW) and Henry's law constant) as is shown in Fig. 1 SM. Although the plots seem to show a relationship between the EOC removal efficiencies and MW (Spearman's correlation coefficient = -0.197), log K_{ow} (Spearman's correlation coefficient = -0.080) and Henry's law constant (Spearman's correlation coefficient = -0.075), no significant correlations were found (significance level >0.05). This may be explained by the complexity of the chemical compounds studied as well as the fact that different removal processes occurred simultaneously. Conversely, a statistically significant relationship between influent concentration and removal efficiency was obtained (Spearman's correlation coefficient = 0.627, significance level =0.002). This may be explained by the fact that biodegradation needs a certain compound concentration before microbial degradation is stimulated. Nevertheless, this general rule must be applied with care and further work is necessary in this field.

The removal efficiency of HRAPs is comparable to that of conventional activated sludge WWTPs,

as can be seen in Table 2 (overall removal efficiencies of 84% and 59% in warm and cold season respectively). Hijosa-Valsero et al. [38] found that waste stabilization ponds (WSPs) were capable of moderately removing pharmaceuticals such as naproxen (33%) and ibuprofen (56%). Matamoros et al. [39] found that unsaturated CWs were capable of removing up to 90% of pharmaceuticals and personal care products such as ibuprofen, naproxen and galaxolide, but did not remove carbamazepine. Hence, HRAPs seem to be as or more efficient than other biological wastewater treatment technologies, such as CWs or WSPs, with regard to EOC removal. Therefore, HRAPs can be considered a suitable technology for the treatment of wastewaters containing EOCs, with the added advantage that they produce microalgal biomass, do not require aeration and have smaller land area requirements than other engineered natural wastewater treatment technologies (i.e. CWs and WSPs). Nevertheless, since the effectiveness of engineered natural wastewater treatment technologies for removing EOCs has been shown to rely on different key design and environmental factors, such as HRT and seasonality [40], the next two sections will explore the influence of these factors on HRAPs.

305

3.2.1. *Effect of hydraulic retention time (HRT)*

HRT is a key design parameter for achieving proper removal efficiency of biodegradable organic contaminants from wastewaters engineered natural treatment systems such as constructed wetlands and waste stabilization ponds [32]. In fact, it has already proved that EOC removal in engineered natural treatment systems and activated sludge WWTPs increases as HRT increases due to the increase of biodegradation and sorption processes [33, 41]. No significant differences in their performance were observed between HRTs in the warm season ($p>0.05$), but significant differences were found in the cold season for those compounds that has already been described in the literature [41] as been removed by biodegradation (i.e. caffeine, 4%; ibuprofen, 7%; methyl dihydrojasmonate, 5%; oxybenzone, 13%; naproxen, 8% and triphenyl phosphate, 44%),

316 photodegradation (i.e. ketoprofen; 25% and triclosan, 20%) and sorption or volatilization (i.e.
317 galaxolide, 24% and tonalide, 16%). As already noted, conventional water quality parameters such
318 as COD and NH₄-N behaved similarly. From these results, it can be postulated that biodegradation,
319 photodegradation, sorption and volatilization removal mechanism were likely affected by the
320 increase of HRT in the cold season. García-Rodríguez et al. [40] reported that biological wastewater
321 treatment technologies for removing EOCs are highly dependent on HRT because it enhances
322 biodegradation, photodegradation and sorption removal processes. In general, the higher the HRT,
323 the greater the EOC removal efficiency. However, our results suggest that an HRT of 4 days is
324 enough to remove most of the compounds in both seasons. Therefore, while this technology is
325 competitive in terms of HRT compared to CWs and WSPs, activated sludge WWTPs are generally
326 set at an HRT of 12-24 hours or lower. Notwithstanding the foregoing, activated sludge WWTPs
327 also have higher energy requirements (0.6 kWh m⁻³ for activated sludge WWTPs vs. 0.02 kWh m⁻³
328 for HRAPs). Finally, the lower microcontaminant sorption onto the biomass (table 1-SM) than in
329 conventional activated sludge WWTPs biosolids [42] is relevant for risk management and sludge
330 valorisation. This low bioaccumulation of microcontaminants into the biomass have already been
331 reported for vegetables [43], but this is the first time that it has been assessed for microalgae.

332

333 3.2.2 Seasonality (environmental conditions)

334 Seasonality is relevant to achieving adequate EOC removal efficiency in mild climates such as that
335 of the NW Mediterranean because it affects temperature, daylight duration and intensity, and
336 biomass production, four important factors influencing biodegradation, photodegradation,
337 volatilization and sorption EOC removal processes [40]. Significant differences (p<0.05) between
338 the warm and cold seasons were observed for the removal of above described biodegradable
339 compounds (i.e. caffeine, ibuprofen, methyl dihydrojasmonate, oxybenzone, naproxen,
340 benzothiazole, methylparaben, benzotriazole, 5-methyl-benzotriazole, OH-benzothiazole, and

341 triphenyl phosphate), photodegradable compounds (i.e. ketoprofen, diclofenac, and triclosan) and
342 highly hydrophobic / moderately volatile compounds (i.e. galaxolide and tonalide). The higher
343 temperature (11 vs. 26 °C, on a daily average) and greater solar radiation (1675 vs. 7049 W d⁻¹ m⁻²)
344 in the warm season may explain these differences. It should be noted that the effect of seasonality
345 on the pollutant removal performance of this technology for the most abundant compounds was low
346 (around 10-20%) or null (i.e. for caffeine, acetaminophen, ibuprofen and methyl dihydrojasmonate).
347 In contrast, various authors [44-46] have reported higher seasonal variability for EOC removal by
348 other engineered natural wastewater treatment technologies (CWs or WSPs). Hence, although the
349 HRAP technology seems to be a robust and reliable wastewater treatment technology in terms of
350 EOC removal efficiency, further studies are required to provide more insight.

351

352 3.3. Aquatic risk assessment

353 Aquatic risk assessment throughout the HRAP treatment was performed based on the
354 concentrations of the detected EOCs in the influent and effluent water samples, and the listed EC50
355 values for *Daphnia magna*. Hazard quotient indexes (HQs) were calculated according to the
356 following equation (2):

$$357 \quad HQ = \frac{MEC}{PNEC} \quad (Equation 2)$$

358

359 where PNEC is the predicted non-effect concentration and MEC is the measured environmental
360 concentration at the influent or effluent of each HRAP reactor. PNEC values were estimated for
361 *Daphnia magna*, dividing the EC50 values (48 hours) by a recommended arbitrary safety factor of
362 1000 [47]. The EC50 values used in this study were collected from the literature and are
363 summarized in Table 2SM. When more than one EC50 value was reported for a single compound,
364 the lowest value was used. When no experimental values were available, the EC50 values were
365 estimated with ECOSAR v1.10 (EPI Suite software, US EPA).

366 Fig. 3 shows the individual HQ for each of the studied EOCs in the HRAP influent and effluent
367 water samples. As the difference between the EOC removal efficiencies at both of the studied HRTs
368 was minimal, the risk assessment was only performed at an HRT of 4 days (under the most critical
369 operating conditions). Acetaminophen, ibuprofen and oxybenzone exhibited higher HQs in influent
370 wastewater samples ($HQ > 1$), mainly due to their high concentration. Following the treatment in the
371 HRAP system, all of the studied EOCs had an $HQ < 1$. The most relevant compounds in the treated
372 wastewater effluents, apart from those observed at the influent, were triclosan and galaxolide. These
373 HQ values for the EOCs are in keeping with those reported in the literature for treated wastewater
374 effluents [41]. In fact, triclosan has already been postulated as a critical compound in terms of
375 contribution and environmental risk in wastewater effluents [48]. Hence, von der Ohe et al. [49]
376 argued that triclosan should be seriously considered as a candidate for regulatory monitoring and
377 prioritization on a European scale on the basis of realistic PNECs.

378 As previously described by various authors, HQs should follow an additive model [6]. Hence, the
379 final HQ for each water sample can be calculated as the sum of each individual HQ. The cumulative
380 HQs for the influent wastewater samples were 8.45 and 6.20 for the summer and winter campaigns,
381 respectively. However, they fell 93% (warm season) and 72% (cold season) following the treatment
382 with the HRAPs. Consequently, in summer the cumulative HQ was less than 1 (0.62), whereas in
383 winter it was significantly higher ($HQ = 1.73$). These results are in keeping with the high reduction in
384 acute toxicity achieved by other biological wastewater treatment technologies, such as CWs [50].
385 Nevertheless, this ecological risk assessment was only performed for the target EOCs; therefore,
386 further studies may be needed to include other EOCs and related transformation products.

387

388 4. Conclusions

389 This study has shown that microalgae-based wastewater treatment systems (such as HRAPs) enable
390 the removal of a wide range of EOCs from urban wastewater. Removal efficiency ranged from none

391 to up to 99%. The EOCs were classified into four groups in accordance with their average removal
392 efficiency in HRAPs: high removal (>90%: caffeine, acetaminophen, ibuprofen, methyl
393 dihydrojasmonate and hydrocinnamic acid), moderate-high removal (from 60% to 90%:
394 oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate,
395 triclosan, bisphenol A and octylphenol), moderate-low removal (from 40 to 60%: diclofenac,
396 benzotriazole, OH-benzothiazole, triphenyl phosphate, cashmeran, diazinon, celestolide and
397 atrazine) and poor or no removal (<30%, carbamazepine, benzothiazole, methyl paraben, tris(2-
398 chloroethyl) phosphate, and 2,4-D). The removal of emerging contaminants in HRAPs was only
399 affected by the HRT during the cold season, whereas no differences were observed in the warm
400 season. The most frequently occurring compounds (caffeine, acetaminophen and ibuprofen) had
401 removal efficiencies of up to 90% that were minimally affected by seasonality and HRT. The
402 ecotoxicological risk assessment study revealed that the HQ for the influent wastewater was
403 removed by up to 90%, indicating no acute toxicity risk associated with the studied EOCs at the
404 water effluents.

405

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411

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Table 1. Average concentration and standard deviation of conventional water quality parameters (n=8 per campaign). Removal efficiencies for COD and NH₄-N are shown in brackets for the two HRAP (4 and 8 d HRT). **COD (chemical oxygen demand); DO (dissolved oxygen); TSS (total suspended solids).**

	Primary effluent	Warm season		Primary effluent	Cold season	
		HRT = 4 d	HRT = 8d		HRT = 4 d	HRT = 8d
Solar radiation (W m ⁻² d ⁻¹)		7347.5±898			1674.6±744	
T (°C)	28±1	25±1	25±1	16±1	13±1	13±1
DO (mg L ⁻¹)	2±1	6±1	8±1	7±2	10±1	12±2
pH	8±1	8±1	9±1	8±1	8±1	9±1
TSS (mg L ⁻¹)	118±112	316±50	346±38	-	110±23	149±15
Biomass production (gTSS m ⁻² d ⁻¹)	-	24±8	13±2	-	8±2	6±0.3
COD (mg L ⁻¹)	156±79	52±9* (75**)	52±12* (84**)	342±107	67±7* (83**)	52±7* (88**)
NH ₄ -N (mg L ⁻¹)	81±9	0.6±0.3(99**)	0.7±0.5(99**)	19±4	2±1 (90**)	0.4±0.1 (98**)

* soluble COD; ** calculation corrected for evaporation water losses.

Table 2. Seasonal removal efficiency (%) of target EOCs in the HRAPs operated at HRT of 4 and 8 days. EOCs have been sorted by their abundance in Fig. 2.

Name	Warm season		Cold season		Activate d sludge WWTPs
	HRT 4 d	HRT 8d	HRT 4d	HRT 8 d	
Caffeine	97±1	98±1	85±2 ^{ab}	91±2 ^{ab}	50-99 ^c
Acetaminophen	99±1	99±1	99±1	99±1	99-100 ^c
Ibuprofen	99±1	99±1	86±4 ^{ab}	93±3 ^{ab}	72-100 ^c
Methyl dihydrojasmonate	99±1	99±1	92±2 ^{ab}	97±1 ^{ab}	98 ^d
Oxybenzone	97±1	99±1	75±10 ^{ab}	88±4 ^{ab}	63-98 ^c
Ketoprofen	87±6 ^b	95±4	50±17 ^{ab}	75±9 ^{ab}	11-100 ^c
Hydrocinnamic acid	99±1	99±1	99±1	99±1	-
5-methyl benzotriazole	83±16	95±8	74±5	77±2 ^a	60 ^e
Naproxen	83±4	89±4	48±5 ^a	60±3 ^a	43-99 ^c
Carbamazepine	46±9	62±15	15±19 ^a	34±15 ^a	<nr-62 ^c
Galaxolide	94±1b	97±1	47±1 ^{ab}	71±2 ^{ab}	88 ^c
Benzothiazole	70±6	78±7	13±8 ^a	30±14 ^a	40-60 ^d
Diclofenac	82±6	92±3	21±29 ^a	29±14 ^a	<0-81 ^c
Methylparaben	59±12	75±8	12±9 ^a	25±11 ^a	82-91 ^h
Benzotriazole	74±7	84±4	33±10 ^a	41±5 ^a	60 ^e
Tonalide	84±1b	90±1	51±5 ^a	67±7 ^a	85 ^c
OH-Benzothiazole	80±3	82±5	20±17 ^a	37±11 ^a	50-70 ^d
Tributyl phosphate	82±5	86±8	69±8	78±2	55-86 ^g
Tris(2- chloroethyl)phosphate	39±28	63±12	15±23	21±19	nr ^c
Triphenyl phosphate	82±2b	89±1	24±6 ^{ab}	68±10 ^{ab}	40 ^g
Triclosan	93±1	95±1	49±5 ^{ab}	69±2 ^{ab}	71-99 ^c
Cashmeran	70±5	79±5	61±3	64±8	50 ^f
Octylphenol	90±6	93±4	58±12 ^a	74±5 ^a	<nr-97 ^c
Diazinon	61±4	63±1	-	-	nr ^c
Celestolide	52±1	53±1	-	-	59 ^f
Atrazine	76±6	85±3	41±7 ^{ab}	69±6 ^{ab}	nr-25 ^c
Bisphenol A	72±14	85±8	66±16	78±6	63-99 ^c
2,4-D	22±10	32±26	-	-	-

^a seasonal statistical difference at p=0.05; ^b HRT statistical difference at p=0.05, ^c [32]; d [51]; d [52]; e [53] ; f[54]; g[55]; h [56]

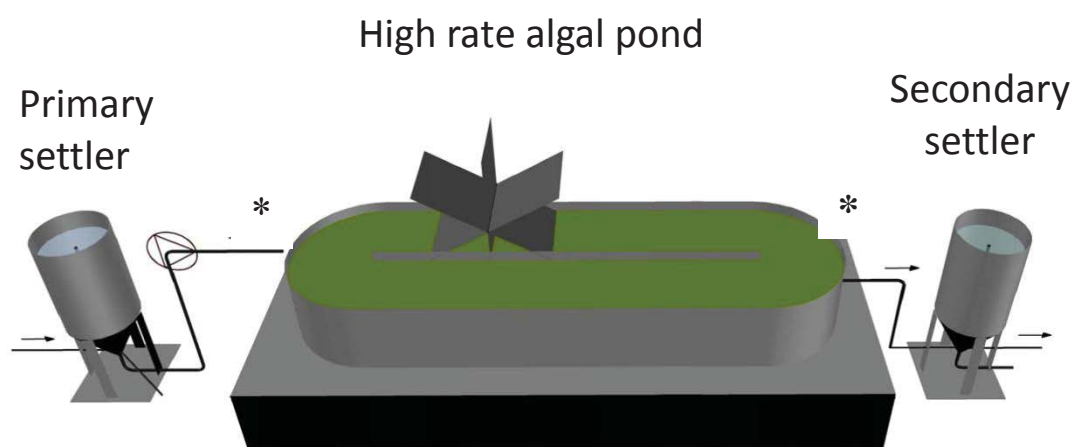


Figure 1. 3D view of treatment units of one line. Primary settler is fed with screened wastewater. Secondary settler allows separation of the biomass produced in the HRAP. Sampling points are indicated (*).

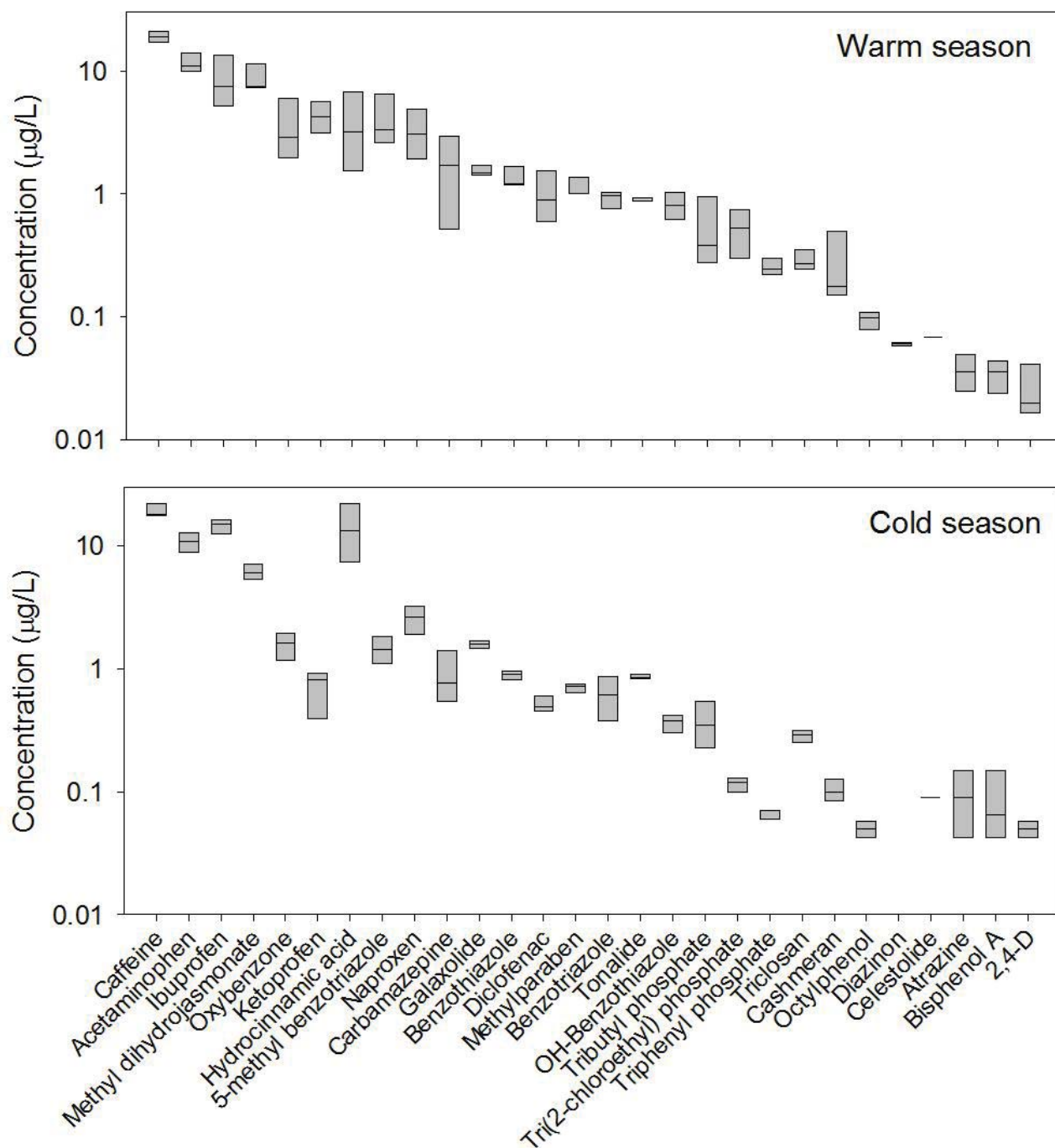


Figure 2. Logarithmic box-plot of the seasonal occurrence of emerging contaminants in the primary effluent wastewater (n=8). The box plots indicate the median, and the 25th and 75th percentiles for each compound. Note that particulate and dissolved phase were both included. Similar profiles were obtained when molar concentrations were compared (not shown).

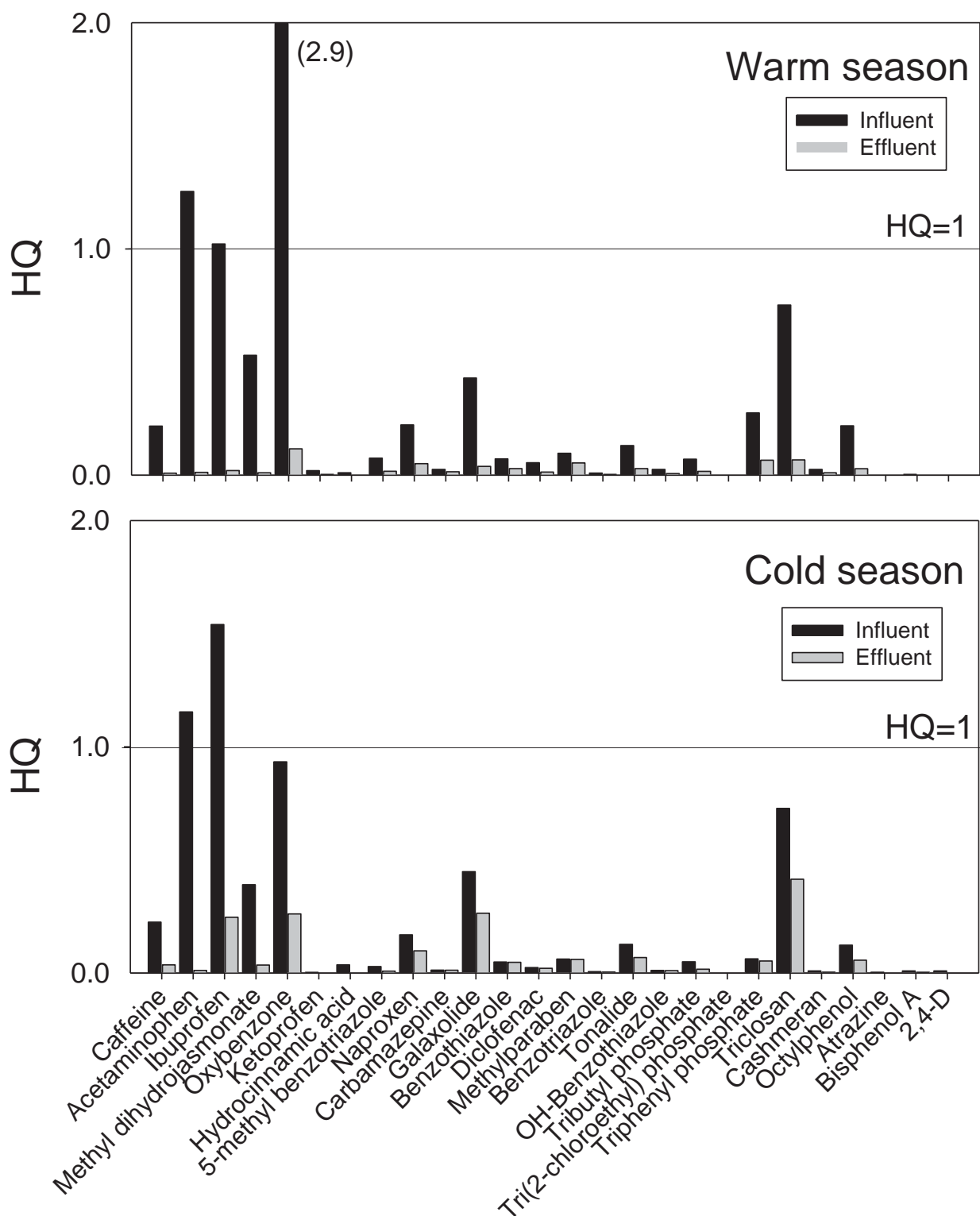


Figure 3. Seasonal hazard quotients (HQs) for the influent and effluent water samples collected from the HRAP set at a HRT of 4days.

Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study

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Number of tables = 2

Number of figures = 1

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Table 1SM. Seasonal occurrence of studied EOCs (ng g⁻¹) in the particulate phase.

Name	Log kow	Warm season		Cold season	
		Influent	Effluent *	Influent	Effluent*
Caffeine	0.16	4.2±1.1	0.1±0.1/0.1±0.2	2±1	0.8±0.3/0.5±0.5
Acetaminophen	0.27	<LOD	<LOD	<LOD	<LOD
Ibuprofen	3.79	3.1±1.2	<LOD	2±0.01	<LOD
Methyl dihydrojasmonate	2.98	2.2±1.0	0.1±0.1/0.1±0.01	0.9±0.2	0.5±0.3/0.1±0.1
Oxybenzone	3.79	8.5±3.2	0.1±0.1/0.2±0.1	5.2±2.1	1.5±1.0/0.1.7±0.9
Ketoprofen	3.00	<LOD	<LOD	<LOD	<LOD
Hydrocinnamic acid	2.29	0.9±0.7	0.1±0.1/0.1±0.1	2.6±0.2	0.1±0.1/0.1±0.1
5-methyl benzotriazole	1.71	<LOD	<LOD	-	<LOD
Naproxen	3.10	1.0±0.1	<LOD	0.3±0.1	<LOD
Carbamazepine	2.25	0.2±0.1	<0.1	0.2±0.1	<0.01
Galaxolide	6.26	12±5.2	0.5±0.1/0.5±0.1	9.2±1.9	3.1±0.2/1.8±0.5
Benzothiazole	2.17	<LOD	<LOD	<LOD	<LOD
Diclofenac	4.02	<LOD	<LOD	<LOD	<LOD
Methylparaben	1.96	1.2±0.7	0.1±0.2/0.2±0.1	0.3±0.3	0.2±0.2/0.1±0.1
Benzotriazole	1.17	<LOD	<LOD	<LOD	<LOD
Tonalide	5.80	7.8±2.5	0.2±0.1/0.2±0.1	5.0±0.5	1.2±0.4/0.6±0.2
OH-Benzothiazole	2.35	<LOD	<LOD	<LOD	<LOD
Tributyl phosphate	3.82	<LOD	<LOD	<LOD	<LOD
Tris(2-chloroethyl)phosphate	1.63	<LOD	<LOD	<LOD	<LOD
Triphenyl phosphate	4.59	0.5±0.2	<LOD	0.2±0.1	0.1±0.1/<LOD
Triclosan	4.66	1.8±0.8	<LOD	0.6±0.5	0.5±0.2/0.3±0.1
Cashmeran	4.49	0.2±0.1	<LOD	0.2±0.1	<LOD
Octylphenol	5.28	0.3±0.1	<LOD	0.2±0.2	<LOD
Diazinone	3.86	<LOD	<LOD	<LOD	<LOD
Celestolide		<LOD	<LOD	<LOD	<LOD
Atrazine	2.82	<LOD	<LOD	<LOD	<LOD
Bisphenol A	3.64	1.2±0.5	<LOD	0.1±0.1	<LOD
2,4-D		<LOD	<LOD	<LOD	<LOD

*First value in the effluent shows concentration at the HRT of 4 days and second value at the HRT of 8 days.

Table 2 SM. EC50 values for *Daphnia magna* (48 hours) used to calculate PNEC. QSAR was used when no experimental data was available.

	EC50 (mg L ⁻¹)	PNEC (µg L ⁻¹) ^a	Reference
Caffeine	46	46	[1]
Acetaminophen	9.2	9.2	[1]
Ibuprofen	9.02	9.02	[1]
Methyl dihydrojasmonate	15.8	15.8	QSAR
Oxybenzone	1.9	1.9	[2]
Ketoprofen	248	248	[3]
Hydrocinnamic acid	398	398	QSAR
5-methyl benzotriazole	51.6	51.6	[4]
Naproxen	15	15	[1]
Carbamazepine	76.3	76.3	[1]
Galaxolide	na	3.5 ^b	[5]
Benzothiazole	19	19	www.env.go.jp
Diclofenac	22	22	[1]
Methylparaben	11.2	11.2	[6]
Benzotriazole	107	107	[4]
Tonalide	na	6.8 ^b	[5]
OH-Benzothiazole	33	33	QSAR
Tributyl phosphate	5.8	5.8	[7]
Tris(2-chloroethyl)phosphate	235	235	[7]
Triphenyl phosphate	1	1	[7]
Triclosan	0.13	0.13	[1]
Cashmaran	11.6	11.6	[2]
Octylphenol			www.environment- agency.gov.uk
	0.27	0.27	
Atrazine	26.3	26.3	[8]
Bisphenol A	10.2	10.2	[9]
2,4-D			EUROPEAN COMMISSION
	100	100	7599/VI/97-final

^a assessment factor (AF) of 1000 was used for EC50 *Daphnia magna* (48 h); ^bNo information about EC50 for *Daphnia magna*, but long term NOECs were available for three tropic levels and therefore a risk assessment factor of 10 was used. When no experimental values were available, the EC50 values were estimated with ECOSAR v1.10 (EPI Suite software, US EPA) which uses the quantitative structure–activity relationship (QSAR) models.

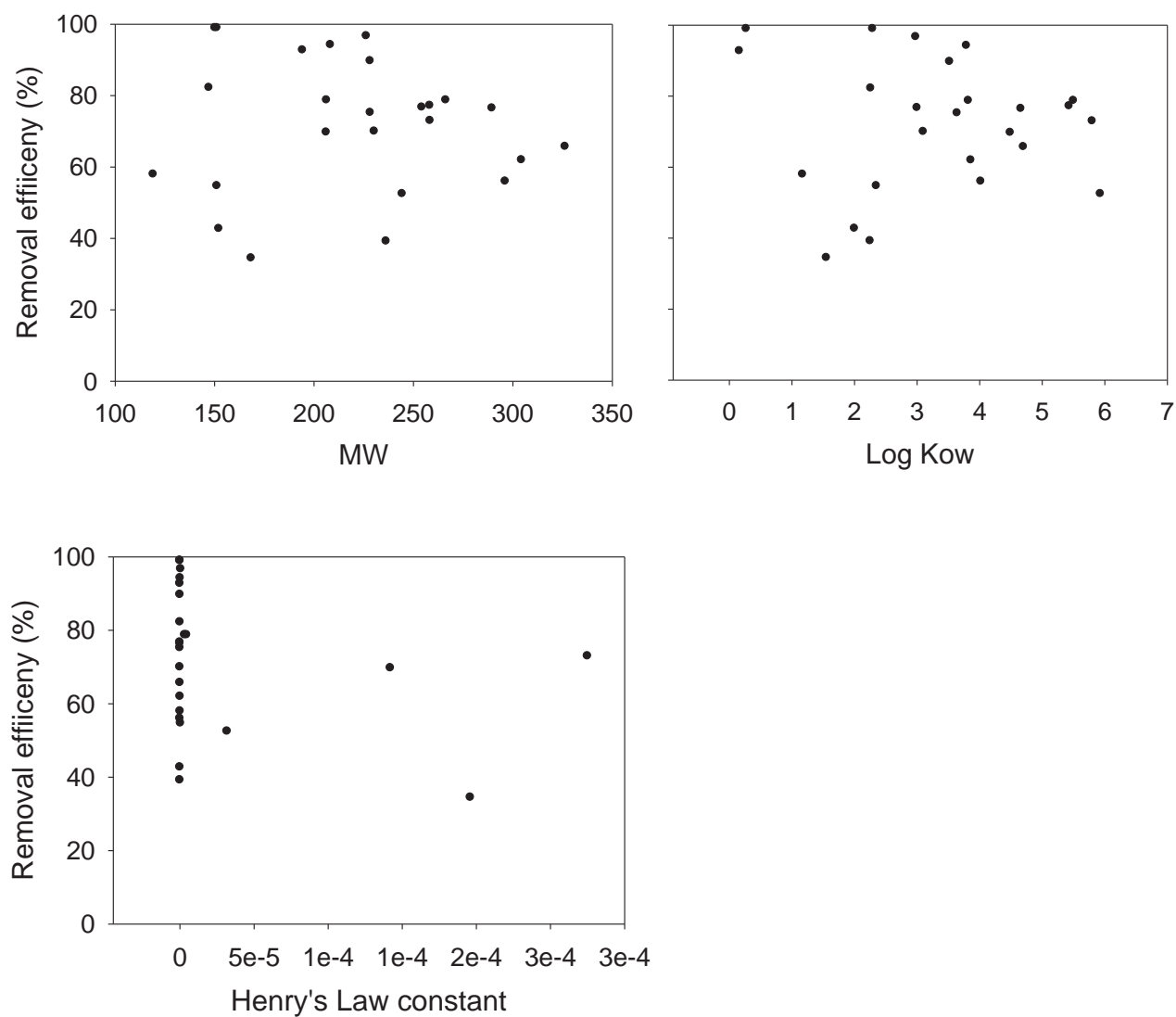


Figure 1 SM. Relationship plots of studied EOC removal against their physical-chemical properties (molecular weight, MW in g mol⁻¹; log Kow; Henry's Law constant in Pa m⁻³ mol⁻¹).

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